Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans

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Non-technical summary We investigated the influence of group III/IV muscle afferents on central motor drive, the development of peripheral locomotor muscle fatigue, and endurance performance time during high-intensity constant-load cycling exercise to exhaustion. Our findings suggest that, on the one hand, afferent feedback ensures adequate circulatory and ventilatory responses to exercise which optimizes muscle O_2 transport and thereby facilitates exercise performance by preventing premature peripheral fatigue. On the other hand, afferent feedback inhibits central motor drive, which is reflected in the restriction of the neural excitation of the locomotor musculature and the reduced tolerance for peripheral muscle fatigue, and thereby limits exercise performance. Taken together, the current investigation revealed the net effects of sensory afferent feedback on time to exhaustion during high-intensity constant-load cycling exercise and showed that intact group III/IV muscle afferent feedback is a vital component in achieving optimal endurance performance.

Abstract We investigated the influence of group III/IV muscle afferents on peripheral fatigue, central motor drive (CMD) and endurance capacity during high-intensity leg-cycling. In a double-blind, placebo-controlled design, seven males performed constant-load cycling exercise (318 ± 9 W; 80% of peak power output (W_{peak})) to exhaustion under placebo conditions and with lumbar intrathecal fentanyl impairing spinal μ-opioid receptor-sensitive group III/IV muscle afferents. Peripheral fatigue was assessed via changes in pre- *vs.* post-exercise quadriceps force in response to supramaximal magnetic femoral nerve stimulation ($\Delta Q_{tw,pot}$). CMD was estimated via quadriceps electromyogram. To rule out a direct central effect of fentanyl, we documented unchanged resting cardioventilatory responses. Compared to placebo, significant hypoventilation during the fentanyl trial was indicated by the 9% lower $\dot{V}_E/\dot{V}_{CO_2}$, causing a 5 mmHg increase in end-tidal P_{CO_2} and a 3% lower haemoglobin saturation. Arterial pressure and heart rate averaged 8 and 10% lower, respectively, during the fentanyl trial and these differences progressively diminished towards end-exercise. Although initially similar, the percent change in CMD was 9 ± 3% higher at end-exercise with fentanyl *vs.* placebo ($P < 0.05$). Time to exhaustion was shorter (6.8 ± 0.3 min *vs.* 8.7 ± 0.3 min) and end-exercise $\Delta Q_{tw,pot}$ was about one-third greater (-44 ± 2% *vs.* -34 ± 2%) following fentanyl *vs.* placebo. The rate of peripheral fatigue development was 67 ± 10% greater during the fentanyl trial ($P < 0.01$). Our findings suggest that feedback from group III/IV muscle afferents limits CMD but also minimizes locomotor muscle fatigue development by stimulating adequate ventilatory and circulatory responses to exercise. In the face of blocked group III/IV muscle afferents, CMD is less inhibited but O_2 transport

compromised and locomotor muscle fatigability is exacerbated with a combined net effect of a reduced endurance performance.

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Abbreviations CMD, central motor drive; CT, contraction time; HR heart rate; iEMG, integrated EMG; ($[La^-]_B$, whole blood lactate concentration; MRFD, maximal rate of force development; MRR, maximal relaxation rate; Q_{tw}, quadriceps single twitch forces; RPE, rating of perceived exertion; $RT_{0.5}$, one-half relaxation time; S_{pO_2}, arterial O_2 saturation was estimated; VL, vastus lateralis; W_{peak}, peak power output.

Introduction

With the onset of exercise, contraction-induced mechanical and chemical stimuli begin to activate molecular receptors on the terminal end of both thinly myelinated (group III) and unmyelinated (group IV) nerve fibres located within skeletal muscle. This activation increases the spontaneous discharge of these thin fibre muscle afferents (Pickar *et al.* 1994; Adreani *et al.* 1997; Kaufman *et al.* 2002; Light *et al.* 2008) which project, via the lumbar dorsal horn of the spinal cord (Wilson & Hand, 1997; Wilson *et al.* 2002), to various sites within the central nervous system (CNS), many of which are currently unknown. The central actions of group III/IV muscle afferents have substantial implications for the exercising human.

First, group III/IV muscle afferents, so-called 'ergoreceptors' (Kniffeki *et al.* 1981; Light *et al.* 2009), are the afferent arm of cardiovascular and ventilatory reflex responses (Kaufman & Forster, 1996) which are mediated via neural circuits in the nucleus tractus solitarii and the ventrolateral medulla (Craig, 1995). For example, when the central projection of group III/IV muscle afferents from the lower limbs is pharmacologically blocked during rhythmic leg exercise, circulation and pulmonary ventilation are substantially compromised. This facilitates arterial hypoxaemia and respiratory acidosis, reduces perfusion pressure, and reduces O_2 delivery to the working muscles – consequences which occur largely independent of the exercise intensity (Amann *et al.* 2010, 2011). Based on these findings, we speculated that sensory feedback from working skeletal muscle might be a vital component in providing a high capacity for rhythmic exercise since muscle perfusion and O_2 delivery determine the fatigability of skeletal muscle and thus affect its performance (Hogan *et al.* 1999; Amann & Calbet, 2008).

Second, group III/IV muscle afferents have been shown to facilitate 'central fatigue' (failure, or unwillingness, of the CNS to 'drive' motoneurons) by exerting inhibitory influences on central motor drive (CMD) during exercise (Gandevia, 2001; Amann, 2011). This has frequently been demonstrated during maximal isometric exercise of a single muscle (e.g. Gandevia *et al.* 1996) and also during high-intensity whole body endurance exercise (Amann

et al. 2008, 2009). Furthermore, via their inhibitory effects on CMD, group III/IV muscle afferents also limit the development of peripheral fatigue during high-intensity endurance exercise to a 'critical threshold' (Amann, 2011). In other words, when peripheral locomotor muscle fatigue reaches a critical level, the intact subject will 'choose' to stop (constant load) endurance exercise or to reduce power output (time-trial exercise) (Amann, 2011). Although some data are available regarding brain areas to which nociceptive muscle afferents are projected (Craig, 2003; Brooks *et al.* 2005), the exact anatomical sites within the CNS mediating the effects of group III/IV muscle afferents on CMD are unknown. However, neural circuits involved in generating motor cortical output 'upstream' from the motor cortex have been implicated (Taylor & Gandevia, 2008; Hilty *et al.* 2011).

Taken together, on the one hand, group III/IV muscle afferents might facilitate endurance performance via optimizing muscle O_2 supply to working muscle; on the other hand, group III/IV muscle afferent feedback might limit endurance exercise performance via inhibiting CMD in order to curtail further development of peripheral fatigue. In recent experiments, we pharmacologically blocked lower limb thin-fibre muscle afferents during a 5 km cycling time trial (Amann *et al.* 2009). An abnormally high power output and performance characterized the first half of the trial with blockade, but this was followed by reduced power output and a slow second half with no *net effect* on overall performance. Based on these observations, which were confounded by providing the opportunity for a changing power output during time-trial exercise, it could be argued that sensory feedback from working locomotor muscle was of no consequence to endurance exercise performance. Although this previous investigation showed significant effects of group III/IV muscle afferents on CMD, the crucial question regarding their effect on endurance exercise performance (and why the improved CMD in the time-trial study was not reflected in a better performance) remained elusive.

The current study was therefore designed to circumvent the confounding effects associated with the availability of a voluntary change in power output (as was the case in the time-trial study) while evaluating muscle afferent blockade-related changes in endurance

performance. Based on this previous work, we now used constant-load endurance exercise combined with measures of quadriceps fatigue to test the following hypotheses: Blocking the central effect of group III/IV muscle afferents during constant-load cycling will attenuate cardiorespiratory responses and compromise O_2 delivery, thereby accelerating the development of peripheral locomotor muscle fatigue. In the human with intact afferent feedback from locomotor muscles, this reduced O_2 transport would, by itself, inhibit CMD and compromise exercise duration, resulting in a level of peripheral fatigue at exercise termination which is comparable to that achieved with normal O_2 delivery (Amann *et al.* 2006*a*, 2007; Amann & Calbet, 2008). However, in the *absence* of feedback from fatiguing locomotor muscles, CMD will not be constrained, resulting in greater muscle fatigue at end-exercise. Accordingly, the enhanced CMD will not be manifested in a sustained power output and exercise performance will be compromised.

Methods

Seven male cyclists volunteered for these studies (age 24.6 ± 2.7 years, body mass 71.2 ± 4.2 kg, height 1.78 ± 0.05 m, maximal O_2 consumption ($\dot{V}_{O_2max}$) 64.7 ± 2.8 ml kg^{-1} min^{-1}). Written informed consent was obtained from each participant. All procedures were approved by the Institutional Review Board and conformed to the *Declaration of Helsinki*.

Protocol

During preliminary visits, all participants were thoroughly familiarized with various procedures involved in this study. Furthermore, each subject performed a maximal incremental exercise test ($20\,W + 25\,W\,min^{-1}$; Amann *et al.* 2004) on a computer-controlled bicycle ergometer (Velotron, Elite Model, Racer Mate, Seattle, WA, USA) for the determination of peak power output (W_{peak}) and maximal O_2 consumption ($\dot{V}_{O_2max}$). On two separate days, double blind and in random order, all subjects performed constant-load bicycle exercise to exhaustion (i.e. pedal frequency below 60% of individual target revolutions per minute for >10 s, despite vocal encouragement) either under placebo conditions including a sham injection of neutral saline into the ligaments between the processus spinosi of the vertebrae at L3–L4 vertebral interspace (placebo), or under experimental conditions including intrathecal fentanyl applied through the same vertebral interspace (fentanyl). The exercise intensity was identical on both days and consisted of 80% of the subjects' W_{peak} (398 ± 18 W). A 5 min warm-up at 2 W (kg body mass)$^{-1}$ preceded the exercise. There was no break between the warm-up and the actual exercise. Throughout exercise,

subjects were instructed to maintain their preferred pedal frequency, as determined during the practice sessions (99 ± 6 rpm). Neuromuscular functions were assessed before and 3 min after exercise. To test for the effects of intrathecal fentanyl on resting quadriceps strength, resting neuromuscular function was assessed before and 5–10 min after its administration (prior to exercise). Each exercise session was separated by at least 72 h and was completed at the same time of day. Subjects were instructed to refrain from caffeine for 12 h and stressful exercise for 48 h before each exercise trial.

Exercise responses

Pulmonary ventilation ($\dot{V}_E$) and gas exchange were measured breath-by-breath at rest and throughout exercise using an open-circuit system including two pneumotachographs (Hans Rudolph, model 3800) (inspiration and expiration) and two Perkin-Elmer mass spectrometers (model 1100) for the analysis of mixed expired and end-tidal gases. Arterial O_2 saturation was estimated (S_{pO_2}) using a pulse oximeter (Nellcor N-595, Pleasanton, CA, USA) with adhesive forehead sensors. Heart rate (HR) was measured from the R-R interval of an electrocardiogram using a three-lead arrangement. Ratings of perceived exertion (RPE) were obtained at rest, 3 min into the exercise and at exhaustion using Borg's modified CR10 scale (Borg, 1998). Arterialized (Finalgon, Boehringer Ingelheim, Germany) capillary blood samples were collected from an earlobe at rest and during exercise for determination of total whole blood lactate concentration ($[La^-]_B$) using an electrochemical analyser (YSI 1500 Sport, OH, USA). Systolic and diastolic blood pressure was manually measured on the left arm at rest and during exercise. Mean arterial pressure (MAP) was calculated as diastolic pressure $+ \,^1/_3 \times$ (systolic pressure–diastolic pressure). To determine a potential diffusion of fentanyl into the systemic circulation, venous blood samples were drawn about 20–30 min after the placebo/fentanyl injection and again immediately after the exercise, i.e. about 40 min post injection. The blood samples were stored and later analysed for fentanyl content via liquid chromatography–tandem mass spectrometry (Huynh *et al.* 2005).

Neuromuscular function

Electromyography. Quadriceps electromyograms (EMG) were recorded from the right vastus lateralis (VL) using monitoring electrodes with full-surface solid adhesive hydrogel (Kendall H59P, Mansfield, MA, USA), with on-site amplification. Electrodes were placed in a bipolar electrode configuration over the middle of the respective muscle belly. The active electrode was placed over the

motor point of the muscle. The recording electrode was moved along the muscle until a good configuration, confirmed by a 'maximal' M-wave (muscle compound action potential) shape, was achieved. The reference electrode was placed over an electrically neutral site. The interelectrode distance was between 20 and 70 mm. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location at subsequent visits. Proper electrode configuration was checked before the beginning of every experiment. To minimize movement artifacts, electrode cables were fastened to the subject's quadriceps using adhesive tape and wrapped in elastic bandage. The electrodes were used to record: (a) magnetically evoked compound muscle action potentials (M-waves) to evaluate changes in membrane excitability; and (b) EMG throughout exercise to estimate central motor drive. The M-wave properties included conduction time (stimulus artifact to peak of M-wave), peak-to-peak amplitude and total area (Katayama *et al.* 2007).

Raw EMG signals corresponding to each muscle contraction during the exercise trials were recorded for later analysis. The EMG signals were amplified and filtered by a Butterworth band-pass filter (BMA-830, CWE, Inc., Ardmore, PA, USA) with a low-pass cut-off frequency of 10 Hz and a high-pass cut-off frequency of 1 kHz. The slope of the filters was –6 dB octave^{-1}. The filtered EMG signals were sampled at 2 kHz by a 16-bit A/D converter (PCI-MIO-16XE-50, National Instruments, Austin, TX, USA) with custom software (Labview 6.0, National Instruments). A computer algorithm identified the onset of activity where the rectified EMG signals deviated by more than 2 standard deviations above the baselines for at least 100 ms. Each EMG burst was inspected to verify the timing identified by the computer. For data analysis, the integral of each burst (integrated EMG (iEMG)) was calculated

$$\mathrm{iEMG}[|m(t)|] = \int_0^t |m(t)|\,\mathrm{d}t$$

where m is the raw EMG signal. As an estimate of the development of peripheral locomotor muscle fatigue during exercise, mean values for iEMG during each muscle contraction (cycle revolution) were calculated, averaged over each 60 s period of the exercise, and normalized to the first minute of exercise.

Despite the fact that surface EMG is frequently used to estimate CMD, it is critical to consider that many factors can influence the signal and these influences could potentially compromise its validity (Enoka & Stuart, 1992; Farina *et al.* 2004). For example, amplitude cancellation and/or significant filtering effects can attenuate increases in motor unit activity measured by surface EMG and this insensitivity might underestimate increases in CMD (Keenan *et al.* 2005). Furthermore, voluntary muscle

contractions might alter the efficacy of corticospinal synapses on motor neurons (Petersen *et al.* 2003; Martin *et al.* 2008); consequently, measures of muscle EMG may under- or overestimate the CMD.

Magnetic stimulation. For a detailed description we refer to previous studies from our laboratory (Amann *et al.* 2006*b*). Briefly, subjects lay semi-recumbent on a table with the right thigh resting in a preformed holder, the knee joint angle set at 90 deg of flexion and the arms folded across the chest. A magnetic stimulator (Magstim 200, The Magstim Company Ltd, UK) connected to a double 70 mm coil was used to stimulate the femoral nerve. The evoked quadriceps twitch force was obtained from a calibrated load cell (Interface, Model SM 1000, Scottsdale, AZ, USA) connected to a non-compliant strap, which was placed around the subject's right leg just superior to the malleoli. To determine whether nerve stimulation was supramaximal, unpotentiated quadriceps single twitch forces (Q_{tw}) were obtained every 30 s at 50, 60, 70, 80, 85, 90, 95 and 100% of maximal stimulator output. The increment in Q_{tw} from 90% to 95% of the stimulator output was $0.73 \pm 0.59\%$ ($P = 0.18$); the increment in Q_{tw} from 95% to 100% of the stimulator output was $0.17 \pm 0.16\%$ ($P = 0.61$). A plateau in baseline Q_{tw} and M-wave amplitudes with increasing stimulus intensities was observed in every subject, indicating maximal depolarization of the femoral nerve. For the evaluation of quadriceps strength, we used six potentiated single twitch forces ($Q_{tw,pot}$) (Kufel *et al.* 2002). Accordingly, we measured quadriceps twitch force 5 s after a 5 s MVC of the quadriceps and repeated this procedure six times such that six $Q_{tw,pot}$ were obtained. Like Kufel *et al.* (2002), we found that the degree of potentiation was slightly smaller after the first and, to a lesser extent, after the second MVC; therefore, we discarded the first two measurements. Activation of the quadriceps during the MVCs was assessed using a superimposed twitch technique (Merton, 1954; Strojnik & Komi, 1998). Briefly, the force produced during a super-imposed single twitch on the MVC was compared to the force produced by the potentiated single twitch delivered 5 s afterward. The assessment procedures were performed before exercise and at 3 min after exercise. We have previously demonstrated that exercise-induced reductions in potentiated twitch force via magnetic agree closely and consistently with both tetanic and paired twitch techniques for assessing low-frequency fatigue and this agreement also held for electrical stimulation techniques (Johnson *et al.* 1993; Babcock *et al.* 2002; Amann *et al.* 2006*a*; Romer *et al.* 2006). Further, $\Delta Q_{tw,pot}$ pre- *vs.* post-exercise has been shown to be reproducible (CV < 7%) upon repeat testing between days (Polkey *et al.* 1996; Kufel *et al.* 2002; Amann *et al.* 2006*a*). Edwards *et al.* (1977), on the other hand, have reported that tetanic low-frequency

stimulation was significantly more reliable than $\Delta Q_{tw,pot}$ in detecting low-frequency fatigue. Peak force, contraction time (CT), maximal rate of force development (MRFD), one-half relaxation time ($RT_{0.5}$) and maximal relaxation rate (MRR) were analysed for all $Q_{tw,pot}$.

Intrathecal fentanyl

A 20-gauge peripheral I.V. catheter was inserted and a bolus of ~500 ml of normal saline was infused to prevent a potential drop in blood pressure. The subjects were placed in the flexed sitting position and the skin and subcutaneous tissue were anaesthetized at the L3–L4 vertebral interspace using 2–4 ml of 1% (10 mg ml^{-1}) lidocaine. In the experimental trial, a 25-gauge, 3.5 inch Pecan (pencil-point) needle was advanced to the sub-arachnoid space. Free flowing cerebral spinal fluid confirmed subarachnoid positioning of the needle tip. A small amount of cerebrospinal fluid was aspirated and 1 ml of fentanyl (0.025 mg ml^{-1}) injected. The needle was then removed and the subjects remained in an upright sitting position to minimize the potential risk of cephalad movement of fentanyl within the cerebrospinal fluid. In the placebo trial the same needle was advanced (L3–L4), but just prior to entering the subarachnoid space 1 ml of preservative-free normal saline was injected. To evaluate the effects of intrathecal fentanyl on resting neuro-muscular function, per cent voluntary muscle activation, MVC force and $Q_{tw,pot}$ were assessed before and after the application of fentanyl. The time between fentanyl delivery and the start of the constant-load exercise was between 20–30 min.

Statistical analyses

A two-way analysis of variance with repeated measures was performed to evaluate differences between the placebo and fentanyl trial. A least-significant difference test identified the means that were significantly different with $P < 0.05$. Results are expressed as the mean ± SEM.

Results

Effects of intrathecal fentanyl on cutaneous hypoaesthesia, venous fentanyl concentration and resting neuromuscular function

Neurological examinations prior to the start of the exercise revealed cutaneous hypoaesthesia to pinprick and cold perception below T2 in all subjects. This was evident by sensory changes on the torso and by the absence of sensory changes on the upper limbs (demarcating C8 and above). No detectable levels of circulating fentanyl were found in systemic venous blood samples, obtained immediately prior to (~30 min post injection) and following (~40 min post-injection) the exercise. Intra-thecal fentanyl had no effect on per cent voluntary muscle activation (96 ± 1% *vs.* 96 ± 1%, $P = 0.59$), MVC force (557 ± 40 N *vs.* 560 ± 38 N, $P = 0.60$) and $Q_{tw,pot}$ (173 ± 12 N *vs.* 172 ± 13 N, $P = 0.63$).

Exercise performance, locomotor muscle fatigue, M-wave properties and iEMG

Figure 1 illustrates individual effects of intrathecal fentanyl on constant-load cycling exercise performance. Time to exhaustion was on average 21 ± 4% ($P < 0.01$) shorter during exercise with fentanyl blockade, with a range of –9 to –40%.

Immediately after each trial, group mean $Q_{tw,pot}$ was reduced from pre-exercise baseline ($P < 0.001$). The reductions in $Q_{tw,pot}$ were significantly greater at exhaustion following the exercise with fentanyl blockade (–44 ± 2%, range –39 to –51%) *vs.* placebo (–34 ± 2%,
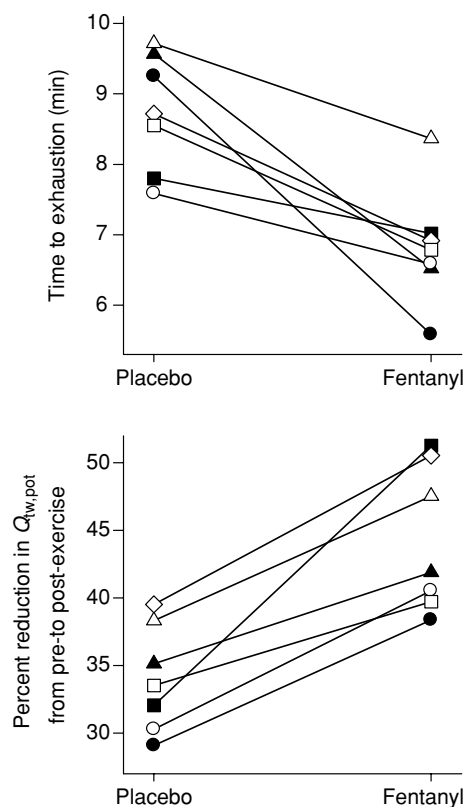

Figure 1. Exercise performance and locomotor muscle fatigue associated with constant load bike exercise (318 ± 9 W) to voluntary exhaustion with (Fentanyl) and without (Placebo) partially blocked neural feedback from locomotor muscles
Top panel illustrates individual differences in time to exhaustion; bottom panel illustrates individual exercise-induced reductions in potentiated quadriceps twitch force ($Q_{tw,pot}$).

Table 1. Effects of constant-load cycling exercise on quadriceps muscle function. Changes in muscle function are expressed as a percent change from pre-exercise baseline. Both exercise trials were performed at the same absolute power output (318 ± 9 W) and until voluntary exhaustion which was reached after 8.7 ± 0.3 min and 6.8 ± 0.3 min under placebo and fentanyl condition, respectively

	Per cent change from pre- to 3 min post-exercise	
	Placebo	Fentanyl
$Q_{tw,pot}$	−34.1 ± 2.0%	−44.1 ± 2.1%*
MRFD	−33.5 ± 3.0%	−44.2 ± 2.6%*
MRR	−34.5 ± 3.1%	−44.4 ± 2.8%*
CT	−6.2 ± 0.5%	−6.2 ± 1.1%
$RT_{0.5}$	6.7 ± 0.7%	8.6 ± 1.0%*
MVC peak force	−9.8 ± 2.0%	−14.1 ± 2.3%*
% voluntary muscle activation	−1.2 ± 0.9%†	−1.7 ± 0.7%
M-wave, peak-to-peak amplitude	1.9 ± 2.0%†	2.1 ± 1.8%†

Values are expressed as means ± SEM. $Q_{tw,pot}$, potentiated single twitch; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; CT, contraction time; $RT_{0.5}$, one-half relaxation time; MVC, maximal voluntary contraction. Per cent muscle activation is based on superimposed twitch technique. Majority of variables changed significantly compared with baseline 3 min after exercise ($P < 0.01$). †Not significantly different from pre-exercise baseline. *$P < 0.05$ *vs.* placebo.

range −30 to −39%) (Table 1 and Fig. 1). MVC force and all within-twitch measurements (MRFD, MRR, CT and $RT_{0.5}$) were altered from baseline immediately after each exercise trial ($P < 0.01$; Table 1). MVC, MRFD, MRR and $RT_{0.5}$ were significantly more reduced after exhaustion under fentanyl *vs.* placebo condition. Following the placebo trial, per cent voluntary muscle activation was similar compared to before exercise (97 ± 1% *vs.* 96 ± 1%, $P = 0.27$). In contrast, per cent voluntary muscle activation was slightly but significantly reduced from
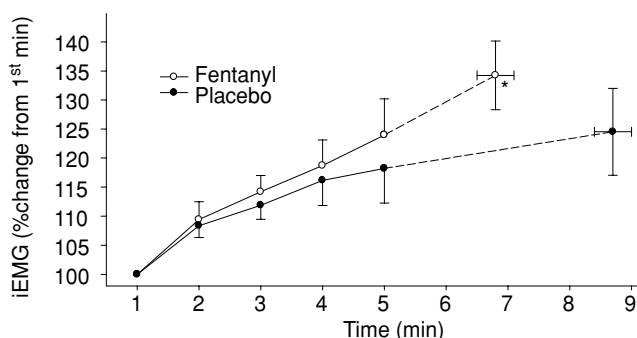

Figure 2. Myoelectrical activity (integrated EMG, iEMG) of vastus lateralis during exercise at 318 ± 9 W
Values are normalized to the mean of the first minute of each trial. *$P < 0.05$ *vs.* end-exercise under placebo condition.

before to 2 min after the fentanyl trial (96 ± 1% *vs.* 95 ± 1%, $P = 0.04$) (Table 1). The rate of development of peripheral muscle fatigue was 67 ± 10% ($P < 0.01$) steeper in the fentanyl *vs.* placebo condition (slope: −6.5 ± 0.3 *vs.* −3.9 ± 0.2, respectively), with individual values ranging between 40% and 118%. Similar to our previous investigation using 5 km cycling time trials (Amann *et al.* 2009), all subjects needed, compared to the placebo trial, assistance in disembarking the ergometer and walking to the bed where post-exercise muscle function was assessed. These observations provide subjective evidence of unusual and severe exhaustion/fatigue.

Membrane excitability was maintained from pre- to post-exercise in all trials as indicated by unchanged M-wave characteristics (peak-to-peak amplitude: placebo: ∼44 mV, $P = 0.56$, fentanyl: ∼45 mV, $P = 0.19$. Area: placebo: ∼58 mV ms^{-1}, $P = 0.87$, fentanyl: ∼56 mV ms^{-1}, $P = 0.46$. Conduction time: placebo: ∼13 ms, $P = 0.14$, fentanyl: ∼13 ms, $P = 0.45$). This suggests that the observed changes in $Q_{tw,pot}$ are mainly due to changes within the quadriceps and that peripheral failure of electrical transmission might be excluded.

Integrated EMG rose significantly from the first minute of exercise to voluntary exhaustion in each of the two conditions (Fig. 2). During the first 4 min of the exercise, iEMG was similar in both conditions ($P > 0.17$). Starting at minute 5, iEMG tended to be higher during the fentanyl trial (4 ± 3%, $P = 0.11$) and was significantly increased by 9 ± 3% (range 1 to 20%) over the placebo condition at end-exercise.

Effects of fentanyl on ventilation, S_{pO_2}, cardiovascular responses and RPE

At rest, fentanyl had no effect on $\dot{V}_E$, breathing pattern, P_{ET,CO_2} or S_{pO_2} (Fig. 3). Fentanyl had a significant overall main effect on $\dot{V}_E$ and $\dot{V}_E/\dot{V}_{CO_2}$ (10 ± 2%) during the first 5 min of exercise and this hypoventilation caused an elevation in P_{ET,CO_2} (14 ± 3%) and a reduction in S_{pO_2} (3 ± 1%) which persisted until exhaustion was reached (Fig. 3 and Table 2). The impact of fentanyl on ventilation and haemoglobin saturation diminished progressively towards the end of exercise; however, $\dot{V}_E/\dot{V}_{CO_2}$ and S_{pO_2} remained significantly lower (7 ± 2% and 1.7 ± 0.6%, respectively) and P_{ET,CO_2} significantly higher (4 ± 1 mmHg) at exhaustion with fentanyl *vs.* placebo (Table 2 and Fig. 3). The lower $\dot{V}_E$ during the fentanyl trial was due to a reduction in breathing frequency (Table 2).

At rest, fentanyl had no effect on HR and MAP (Figs 3 and 4). Fentanyl had a significant overall main effect on HR during the first 5 min of exercise (Fig. 3). However, the impact of fentanyl diminished progressively towards the end of exercise and HR was similar at exhaustion in both

conditions (Table 2 and Fig. 3). MAP was significantly lower at minute 3 during the exercise ($6 \pm 2\%$, range 1 to 10%) and this discrepancy persisted until exhaustion ($8 \pm 2\%$, range 2 to 13%; $P < 0.05$) (Fig. 4).

RPE at the third minute of exercise was $27 \pm 2\%$ (range 0 to 67%; $P < 0.05$) lower in the fentanyl condition compared to placebo; at end-exercise, there was no significant difference in RPE between the two conditions (Table 2).

Discussion

We investigated the influence of group III/IV muscle afferents on CMD (as estimated from changes in quadriceps EMG), the development of peripheral locomotor muscle fatigue, and endurance performance time during high-intensity constant-load cycling exercise to exhaustion. In the absence of the central projection of lower limb muscle afferents, CMD (i.e. output of spinal
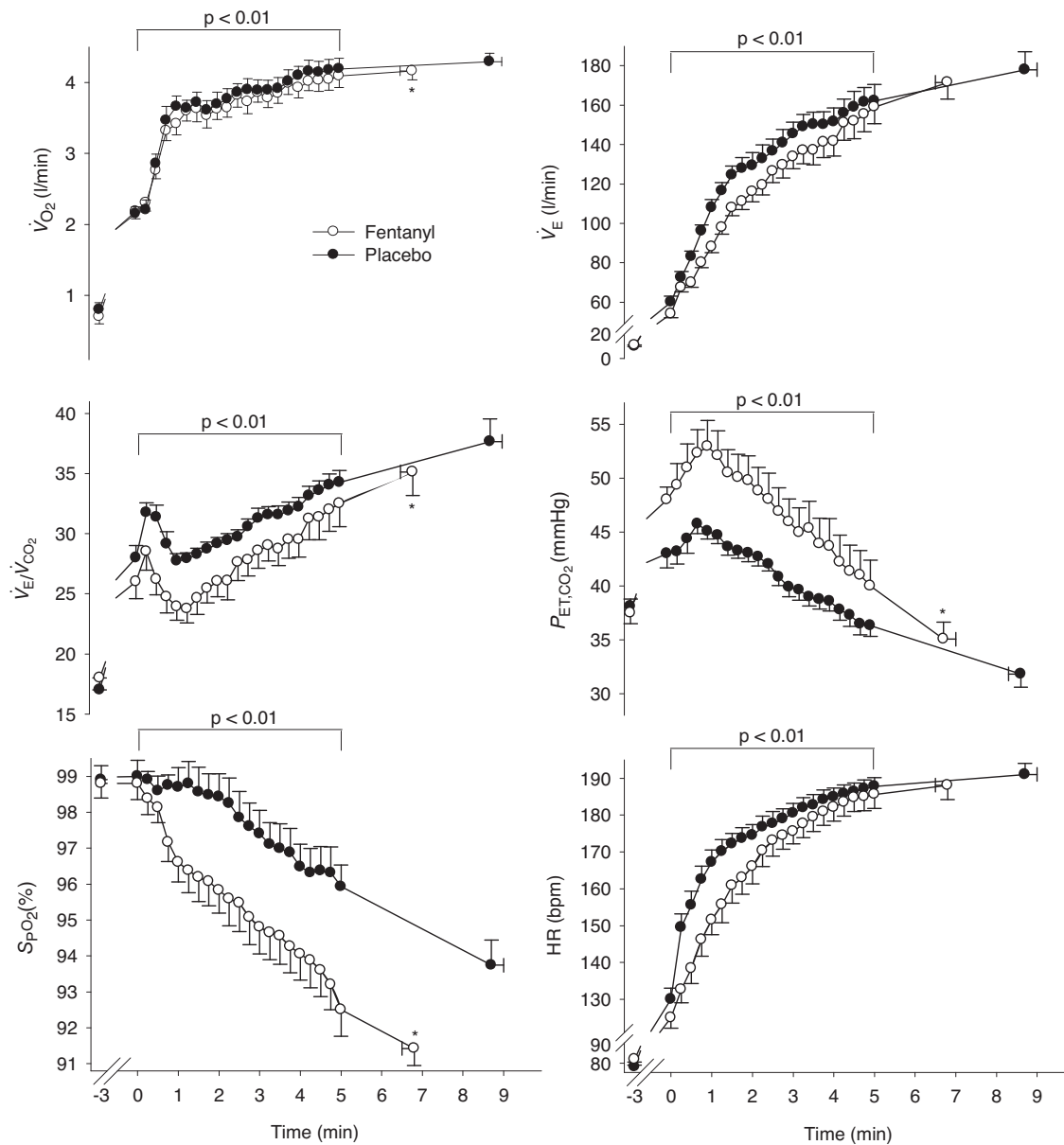

Figure 3. Metabolic and cardioventilatory responses during a brief rest period and during constant load bike exercise (318 ± 9 W) to exhaustion with (Fentanyl) and without (Placebo) partially blocked neural feedback from locomotor muscles
The *P* value indicates the overall main effect of fentanyl during the first 5 min of exercise. *$P < 0.05$ *vs.* end-exercise under placebo condition.

Table 2. Mean physiological response to the third and the final minute of constant load bike exercise to exhaustion

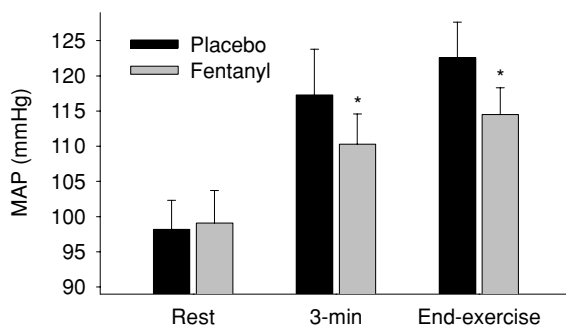|	Placebo		Fentanyl	
	Mean over 3rd min	Mean over final min	Mean over 3rd min	Mean over final min
Power output (W)	318 ± 9	318 ± 9	318 ± 9	318 ± 9
Exercise time (min)	—	8.7 ± 0.3	—	6.8 ± 0.3*
HR (beats min^{-1})	179 ± 3	191 ± 3	173 ± 4*	188 ± 4
$\dot{V}_E$ (l min^{-1})	139 ± 6	178 ± 9	125 ± 6*	172 ± 8
f_R (breaths min^{-1})	42.7 ± 3.1	57.5 ± 2.6	40.9 ± 3.2	55.7 ± 1.8
V_T (l)	3.1 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	3.0 ± 0.2
$\dot{V}_{O_2}$ (l min^{-1})	3.85 ± 0.14	4.29 ± 0.12	3.79 ± 0.14	4.17 ± 0.13*
$\dot{V}_{CO_2}$ (l min^{-1})	4.52 ± 0.17	4.73 ± 0.12	4.55 ± 0.19	4.90 ± 0.17*
$\dot{V}_E/\dot{V}_{O_2}$	36.0 ± 1.0	41.5 ± 1.8	33.0 ± 1.2*	41.2 ± 1.7
$\dot{V}_E/\dot{V}_{CO_2}$	30.3 ± 0.6	37.7 ± 1.9	27.2 ± 1.4*	35.1 ± 2.0*
$P_{ET}O_2$ (mmHg)	106.4 ± 1.3	111.4 ± 1.5	102.6 ± 1.3*	110.4 ± 1.1
P_{ET,CO_2} (mmHg)	41.4 ± 0.8	31.8 ± 1.2	47.4 ± 2.1*	35.0 ± 1.6*
S_{pO_2} (%)	97.8 ± 0.7	93.7 ± 0.7	95.2 ± 0.8*	92.1 ± 0.5*
RPE [a]	4.0 ± 0.2	9.2 ± 0.3	3.0 ± 0.4*	8.9 ± 0.4
[La$^-$]$_B$ (mmol l^{-1}) [a,b]	6.4 ± 0.3	13.1 ± 0.8	6.6 ± 0.4	14.4 ± 0.8

[a]Taken at minute 3 and at end exercise; [b]$n = 6$; *$P < 0.05$ *vs.* placebo.

motoneurons) was increased but the cardiovascular and ventilatory responses were compromised and peripheral fatigue accumulated nearly 70% faster compared to the placebo condition. Despite the greater CMD at exhaustion with fentanyl, the additional 'drive' was not reflected in a better endurance performance compared to placebo exercise. Quite the contrary, in the fentanyl trial, the subjects actually terminated their exercise about 2 min (or 21%) prematurely (*vs.* placebo) and despite this shorter exercise duration all subjects experienced a substantially greater degree of end-exercise peripheral locomotor muscle fatigue. As a consequence of this severe degree of peripheral fatigue, we speculate that the locomotor muscles eventually failed to respond appropriately to the enhanced neural excitation and, *despite the higher CMD,*

were no longer capable of generating the required power output and exercise performance time was compromised.

Fentanyl site of action

To attenuate the central projection of lower limb muscle afferents, we used lumbar intrathecal fentanyl to block the spinal transmission of μ-opioid receptor-sensitive muscle afferents (Wilson & Hand, 1997). We excluded a diffusion of fentanyl into the systemic circulation or a migration of the drug beyond the cervical level based on the following observations. First, cutaneous hypoaesthesia to pinprick and cold perception was evaluated immediately prior to the beginning of the exercise with fentanyl blockade and occurred below T2 in all subjects. Second, identical resting MAP, HR, $\dot{V}_E$ and P_{ET,CO_2} were observed in both the placebo and the fentanyl conditions. Third, no detectable level of fentanyl was found in systemic venous blood samples taken immediately prior to, and again ~10 min after the exercise. Finally, previous experiments also showed that the ventilatory response to inhaled CO_2 was preserved following intrathecal fentanyl, which speaks against the migration of the drug to the level of the brainstem (Amann *et al.* 2010, 2011; Hilty *et al.* 2011).

Figure 4. Mean arterial pressure
Mean arterial blood pressure (MAP) at rest, after 3 min and at exhaustion (8.7 ± 0.3 min and 6.8 ± 0.3 min for Placebo and Fentanyl, respectively) following constant load bike exercise at 318 ± 9 W.

Contribution of muscle afferents to locomotor muscle fatigue, CMD, 'tolerance' of peripheral fatigue and exercise performance

Why did blocking group III/IV locomotor muscle afferents cause a 70% greater rate of development of locomotor muscle fatigue during constant load exercise? This

observation is probably explained by the reduced muscle O_2 transport (arterial O_2 content × leg blood flow) attending afferent blockade. Present findings are consistent with recent studies which show that blocking group III/IV muscle afferents resulted in hypoventilation, arterial hypoxaemia, respiratory acidosis and reduced limb blood flow during exercise (Kaufman & Forster, 1996; Amann *et al.* 2010, 2011) – all of which are known to enhance the rate of development of muscle fatigue (Vianna *et al.* 1990; Mador *et al.* 1997; Hepple, 2002; Amann & Calbet, 2008).

Why did blockade of group III/IV locomotor muscle afferents enhance CMD, as indicated by the increased rate of rise in quadriceps EMG during the latter phases of constant load exercise? First, these findings are consistent with those recently reported during time-trial exercise in which afferent blockade elevated quadriceps EMG (Amann *et al.* 2008, 2009) and power output selected by the subject in the early stages of the time trial (Amann *et al.* 2009). We interpret these effects of afferent blockade to mean that in the intact subject, locomotor muscle afferents normally impose an inhibitory influence on CMD; thus, in their absence, the exercising human is willing to 'push harder' (as with time-trial exercise) or longer (as with constant-load exercise).

What are the influences of group III/IV locomotor muscle afferents on the level of peripheral fatigue accumulated at exercise termination, i.e. the critical threshold? In *intact subjects* when the rate of peripheral fatigue development was accelerated by either reducing fraction of inspired O_2 (F_{IO_2}) and S_{aO_2} (Amann *et al.* 2006*a*, 2007; Katayama *et al.* 2007; Romer *et al.* 2007) or imposing pre-exercise fatigue (Amann & Dempsey, 2008; Gagnon *et al.* 2009), the exercise time to exhaustion was also reduced (constant-load exercise) or power output was reduced and performance time was slowed (time trial) – but the level of quadriceps fatigue (% $\Delta Q_{tw,pot}$) incurred at end-exercise remained unchanged from control trials. However, when lower limb muscle afferents were blocked, we found that the level of locomotor muscle fatigue accumulated at the termination of constant load exercise averaged about 40% greater (*vs.* placebo) (Table 1) – which is strikingly similar to the excessive peripheral fatigue previously observed at the termination of time-trial exercise with afferent blockade (Amann *et al.* 2009). This 'excessive' muscle fatigue resulted in significant, sustained impairment of ambulation in the post-exercise period. We interpret these effects of afferent blockade to mean that the CNS processes group III/IV locomotor muscle afferent feedback in order to limit CMD and thus confines the development of peripheral fatigue to a critical threshold beyond which the associated sensory input would not be

tolerable for long periods (Gandevia, 2001; Amann, 2011). Accordingly, our finding that greater peripheral fatigue accumulated at end-exercise when muscle afferents were blocked, must mean that exercise continued (at least for a limited period – see below) beyond the achievement of this critical fatigue threshold and in the presence of compromised muscle O_2 transport, when feedback was not available to inform the CNS that deleterious conditions existed in the working muscle.

Why did blockade of group III/IV locomotor muscle afferents reduce endurance performance? The overall rate of peripheral fatigue development averaged 70% faster during the exercise with fentanyl blockade *vs.* placebo. As a consequence, the significantly higher CMD towards the end of exercise with fentanyl blockade (*vs.* placebo) was confronted with a substantially more fatigued locomotor musculature (Fig. 1). Apparently then, the gain in CMD was not sufficient to overcome the severe degree of peripheral fatigue and, despite the higher CMD, the locomotor muscles were no longer able to continue to generate the required power output (~318 W) – at least for as long a period as they did with intact afferents when O_2 transport was normal (also see above). Stated differently, the extra CMD, resulting from the attenuated inhibitory effects of group III/IV muscle afferents, could not be 'translated' into sustained power output and a better endurance performance. This supports an earlier animal study which showed that treadmill running time to exhaustion was shorter in rats with abolished group III/IV muscle afferent feedback as compared to control animals (Dousset *et al.* 2004). So, although blocking μ-opioid receptor-sensitive muscle afferents during exercise lessened the inhibition of CMD, the missing feedback also attenuated cardioventilatory responses (Table 2, Figs 3 and 4) thereby negatively impacting the subjects' locomotor muscle fatigue and endurance capacity. In other words, the positive effect of attenuating CMD inhibition was outweighed by the negative effect of attenuating circulatory and ventilatory response to exercise, with the overall net effect of a 21% average reduction in endurance time to exhaustion.

Conclusion

The current findings using constant-load exercise and our previous work with time-trail exercise revealed a crucial twofold role of group III/IV locomotor muscle afferents in the exercising human. On the one hand, afferent feedback ensures adequate circulatory and ventilatory responses to exercise which optimizes muscle O_2 transport and thereby facilitates exercise performance by preventing premature fatigue at the level of the locomotor muscles. On the other hand, afferent feedback inhibits

CMD (i.e. facilitates central fatigue), which is reflected in the restriction of the neural excitation of the locomotor musculature and the reduced tolerance for peripheral muscle fatigue, thereby limiting exercise performance. The current investigation now revealed the net effects of sensory feedback as a 'double-edged sword' (Amann & Secher, 2010) on time to exhaustion during high-intensity constant-load cycling exercise and showed that intact group III/IV muscle afferent feedback is a vital component in achieving optimal endurance performance.

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